

Appl. No. 09/719,867
Amdt. Dated October 22, 2004
Reply to Office Action of August 27, 2004

Amendments to the Claims:

This listing of claims will replace all prior versions, and listing, of claims in the application:

Listing of Claims

Claim 1 (previously presented): A process for identifying mutations that confer resistance to a compound comprising:

- a) generating a defined set of overlapping PCR products, of approximately 10 kb to approximately 15 kb, containing random point mutations which encompass the complete chromosome of a wild-type strain of bacteria for which the chromosomal sequence is known;
- b) transforming pools of the PCR products from step (a) corresponding to about 100 kb of the chromosome into the wild-type strain and isolating the resulting strains that are resistant to a compound;
- c) transforming the wild-type strain with the individual PCR products from a resistant strain isolated in step (b) and isolating the resulting strains that are resistant to the compound;
- d) generating smaller PCR products of approximately 1 to approximately 4 kb which encompass the PCR product from a resistant strain identified in step (c);
- e) transforming a wild-type strain with one of the smaller PCR products from step (d) and determining whether the strain is resistant to the compound;
- f) repeating step (e) for each of the smaller PCR products until a strain resistant to the compound is found or until all of the smaller PCR products have been evaluated;
- g) sequencing the smaller PCR product isolated from a strain resistant to the compound and comparing the sequence to the corresponding sequence in a wild-type strain to determine the mutation or mutations that confer resistance to a compound.

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Claim 2 (cancelled)

Claim 3 (withdrawn): Mutations in *Neisseria gonorrhoeae* GyrA associated with quinolone resistance selected from: Asp90 to Glu, Ser91 to Cys, Asp95 to His, Glu161 to Gly, Glu161 to Lys, Asn65 to His, Asp80 to Gly, and Glu62 to Lys.

Claim 4 (original): The process according to Claim 1 for identifying and characterizing drug-target interactions.

Claim 5 (previously presented): A process for identifying and characterizing mutations that confer resistance to a compound comprising:
generating overlapping PCR products of approximately 10 kb to approximately 15 kb which encompass the complete chromosome from a wild-type bacteria strain for which the chromosomal sequence is known, under conditions that allow for mutation of the fragments;
allowing one or more of the generated PCR products to be incorporated into the chromosome of wild-type bacteria;
isolating bacterial strains that demonstrate resistance to a compound; and
identifying the mutation responsible for the resistance.

Claim 6 (previously presented): A process for identifying mutations that confer resistance to a compound comprising:

- a) generating overlapping PCR products of approximately 10 kb to approximately 15 kb which encompass the complete chromosome from a strain of bacteria which demonstrates resistance to a compound;
- b) allowing one or more of the generated PCR products to be incorporated into the chromosome of a wild-type bacteria;
- c) isolating bacterial strains that demonstrate resistance to the compound; and identifying the mutation responsible for the resistance.

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Claim 7 (cancelled)

Claim 8 (original): The process of claim 1 where the bacteria is from the group of genus *Neisseria*, *Haemophilus*, *Streptococcus*, *Staphylococcus*, or *Escherichia*.

Claim 9 (original): The process of claim 5 or 6 where the bacteria is from the group of *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, or *Escherichia coli*.

Claim 10 (previously presented): The process of claim 5 where the compound is a fluoroquinolone.

Claim 11 (previously presented): The process of claim 5 where the compound is ciprofloxacin.

Claim 12 (previously presented): The process of claim 5 where the compound is clinafloxacin.

Claim 13 (previously presented): The process of claim 5 where the compound is dihydroxydiphenylether (DHDPE).

Claim 14 (previously presented): The process of claim 5 where the compound is Triclosan.

Claim 15 (cancelled)

Claim 16 (previously presented): The process of claim 1 in which the compound inhibits the growth or survival of the wild-type bacteria under any condition.

Claim 17 (previously presented): The process of claim 1 in which the compound inhibits the growth or survival of the wild-type bacteria in culture.

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Claim 18 (previously presented): The process of claim 1 in which the compound inhibits the growth or survival of the wild-type bacteria in an animal host.

Claim 19 (previously presented): The process of claim 1 in which the compound is an inhibitor of type II topoisomerases.

Claim 20 (previously presented): The process of claim 1 in which the compound is an inhibitor of FabI.

Claim 21 (previously presented): The process of claim 1 in which the compound is an inhibitor of enzymes involved in fatty acid biosynthesis.

Claim 22 (previously presented): The process of claim 6 in which a strain of bacteria which demonstrates resistance to a compound was isolated from a culture that had been treated with a chemical mutagen.

Claim 23 (previously presented): The process of claim 6 in which a strain of bacteria which demonstrates resistance to a compound was isolated from a culture that had been treated with ultraviolet light.

Claim 24 (previously presented): The process of claim 6 in which a strain of bacteria which demonstrate resistance to a compound was isolated from a culture that had been subjected to a mutagenic protocol that consisted of insertion of DNA into the chromosome of the bacteria.

Claim 25 (withdrawn): Bacteria comprising a protein in which a contiguous stretch of 40 amino acids is at least 30% identical to residues 75 to 114 of the *Neisseria gonorrhoeae* GyrA and the residue analogous to:
62 is lysine or
63 is arginine or glutamic acid or
65 is histidine or
135 is valine or
161 is glutamic acid or lysine or glycine.

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Claim 26 (withdrawn): *Escherichia coli* strains comprising a GyrA protein in which the amino acid analogous to the *Neisseria gonorrhoeae* GyrA amino acid
62 is lysine or
63 is arginine or glutamic acid or
65 is histidine or
135 is valine or
161 is glutamic acid or lysine or glycine

Claim 27 (withdrawn): *Neisseria gonorrhoeae* strains comprising a GyrA protein in which the amino acid residue
62 is lysine, or
63 is arginine or glutamic acid, or
65 is histidine, or
80 is alanine or glycine, or
90 is arginine or glutamic acid, or
91 is tyrosine or alanine or cysteine, or
92 is proline, or
95 is arginine or alanine or valine or tyrosine or histidine or glycine, or
114 is histidine, or
135 is valine, or
161 is glutamic acid or lysine or glycine.

Claim 28 (withdrawn): *Neisseria gonorrhoeae* strains selected from the group consisting of NG-2707, GC318, NG-2721, NG-2711, NG-2706, NG-2717, NG-2687, GC158, NG-2690, GC219, GC291, NG-2691, NG-2720, NG-2723, GC156, NG-2698, NG-2709, NG2716, NG-2719, and NG-2712.

Claim 29 (withdrawn): A protein comprising in which a contiguous stretch of 40 amino acids is at least 30% identical to residues 75 to 114 of the *Neisseria gonorrhoeae* GyrA and the residue analogous to:
62 is lysine or
63 is arginine or glutamic acid or

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65 is histidine or
135 is valine or
161 is glutamic acid or lysine or glycine.

Claim 30 (withdrawn): *Neisseria gonorrhoeae* GyrA protein comprising amino acid
substitutions when residue

62 is lysine, or
63 is arginine or glutamic acid, or
65 is histidine, or
80 is alanine or glycine, or
90 is arginine or glutamic acid, or
91 is tyrosine or alanine or cysteine, or
92 is proline, or
95 is arginine or alanine or valine or tyrosine or histidine or glycine, or
114 is histidine, or
135 is valine, or
161 is glutamic acid or lysine or glycine.

Claim 31 (withdrawn): Bacteria comprising a protein that is at least 30% identical to the
sequence of the *Neisseria gonorrhoeae* FabI protein in which the amino acid
residue corresponding to

15 is valine, or
20 is threonine, or
23 is glycine, or
25 is valine, or
51 is threonine, or
91 is threonine, or
93 is cysteine or serine, or
95 is valine, or
104 is leucine, or
105 is histidine, or
144 is valine, or

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147 is histidine, or
159 is alanine, or
160 is isoleucine, or
162 is valine, or
193 is asparagine or valine, or
201 is valine, or
203 is tyrosine or valine, or
204 is serine or leucine or isoleucine or valine, or
212 is threonine or valine, or
247 is asparagine.

Claim 32 (withdrawn): A *Neisseria gonorrhoeae* strain selected from the group consisting of NG-2669, NG-2654, NG-2651, NG-2670, NG-2660, NG-2641, NG-2639, NG-2638, NG-2640, NG-2648, NG-2657, NG-2656, NG-2653, NG-2658, NG-2663, NG-2642, NG-2671, NG-2652, NG-2661, NG-2644, NG-2667, NG-2665, NG-2655, NG-2643, NG-2666, NG-2664, NG-2647, NG-2646, NG-2650, NG-2649, NG-2645, NG-2659, NG-2662, and NG-2672.

Claim 33 (withdrawn): An *Escherichia coli* strain comprising a FabI protein with the amino acids analagous to the ones described in claim 31 with the exception of mutations resulting in changing residue
93 to alanine or serine or cysteine or valine,
159 to threonine or,
203 to leucine.

Claim 34 (withdrawn): A protein comprising at least 30% identical to the sequence of the *Neisseria gonorrhoeae* FabI protein in which the amino acid residue corresponding to
15 is valine, or
20 is threonine, or
23 is glycine, or
25 is valine, or

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51 is threonine, or
91 is threonine, or
93 is cysteine or serine, or
95 is valine, or
104 is leucine, or
105 is histidine, or
144 is valine, or
147 is histidine, or
159 is alanine, or
160 is isoleucine, or
162 is valine, or
193 is asparagine or valine, or
201 is valine, or
203 is tyrosine or valine, or
204 is serine or leucine or isoleucine or valine, or
212 is threonine or valine, or
247 is asparagine.

Claim 35 (withdrawn): A *Neisseria gonorrhoeae* FabI protein comprising the amino acid corresponding to residue:

15 is valine, or
20 is threonine, or
23 is glycine, or
25 is valine, or
51 is threonine, or
91 is threonine, or
93 is cysteine or serine, or
95 is valine, or
104 is leucine, or
105 is histidine, or
144 is valine, or

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147 is histidine, or
159 is alanine, or
160 is isoleucine, or
162 is valine, or
193 is asparagine or valine, or
201 is valine, or
203 is tyrosine or valine, or
204 is serine or leucine or isoleucine or valine, or
212 is threonine or valine, or
247 is asparagine.

Claim 36 (previously presented): A process of screening compounds for antibacterial activity comprising:

- a) identifying the mutation that confers resistance to a compound using the method of Claim 1, Claim 5, or Claim 6;
- b) contacting a strain of bacteria containing the mutation with a compound; and
- c) evaluating the compound for antibacterial activity.